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THE ESTIMATION OF ACTIVATED HUMAN BLOOD COAGULATION FACTOR VII

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SUMMARY

The specificity of tissue thromboplastin towards factor VII is such that, with bovine thromboplastin, unactivated human factor VII will not show up in a one-stage test. This property is used to develop a test which is specific for activated factor VII.

INTRODUCTION

Blood coagulation can be triggered by material from wounded tissue. These materials are called tissue thromboplastins. They comprise at least a phospholipid and a protein component, intimately linked together. Thromboplastin activates the plasma protein factor VII. Activated factor VII converts the proenzyme factor X into its enzymatically active form (factor X_a). Factor X_a and factor V adsorb onto a phospholipid–water interface and thus form the prothrombin activating enzyme[1].

Combination of factor VII with (components from) tissue thromboplastin is an essential part in the generation of factor X converting activity[2–4]. There are many indications that combination of factor VII with tissue lipoproteins is not the sole process leading to such an activity. Changes in the factor VII molecule seem to play a role as well. Under various experimental conditions excluding a reaction between factor VII and tissue lipoproteins, factor VII can still become more active in a coagulation assay. Such a change in reactivity will be called activation. Activation has been demonstrated by the action of factor XII (ref. 5), by the action of the kinin system[6] and during purification procedures[7].

It has been demonstrated that activated factor VII can be inhibited by serine esterase inhibitors[8], whereas plasmatic factor VII is not. It is conceivable that, in analogy to the factors IX and X, factor VII plays its biological role when forming part of a lipid–protein complex but also has to be converted from a proenzyme (factor VII) into an enzyme (factor VII_a)[9]. When this is true, tissue thromboplastin would

not only catalyze the proenzyme–enzyme conversion (activate factor VII) but also provide lipoproteins which combine with the activated factor VII so as to form a factor X activator. For a further study of these problems it would be most useful to be able to estimate the activated but uncomplexed form of factor VII and the unactivated form separately.

There is a well founded practical interest in studying the level of activated factor VII in the circulation as well: atherosclerotic lesions offer to the circulating plasma both a foreign surface and a damaged tissue. It is conceivable therefore that these lesions activate circulating factor VII. The fraction of factor VII that circulates in its activated form then would be an indication of the extent of the lesions. The first event in the generation of thrombin by a lesion could be the local concentration of factor VII_a surpassing a critical threshold. The concentration of factor VII_a in the circulation could therefore be an indication of the risk of thrombosis.

MATERIALS AND METHODS

Bovine blood was obtained by jugular venapuncture from normal cows and collected on citrate (final concentration of 10 m*M*). The blood was centrifuged (10 min, 3000 × *g*), the plasma collected and again centrifuged (30 min, 12,000 × *g*) to bring down the remaining platelets. Bovine brain thromboplastin was prepared in a way completely analogous to the preparation of human brain thromboplastin according to Owren and Aas[10]. All other materials and methods are described in refs. 11 and 12.

RESULTS

Table I shows the coagulation times obtained when dilutions of human or bovine plasma are used as a source of factor VII in a factor VII-deficient medium,

TABLE I

FACTOR VII DETERMINATION IN HUMAN, BOVINE AND CROSSED SYSTEMS

Coagulation times obtained with human or bovine plasmas and human or bovine thromboplastins (thr. pl.) in a reagent specific for factor VII containing 27% human factor X, 30% bovine factor V, 31% human factor II and 1.2 mg/ml factor I. The figures are the means of 32 estimations. S.D. ≤ 1%.

Plasma concentration (% v/v)	*Coagulation times (sec)*			
	Human plasma		*Bovine plasma*	
	Human thr. pl.	*Bovine thr. pl.*	*Human thr. pl.*	*Bovine thr. pl.*
10	30.8	66.7	62.6	34.2
5	35.8	73.1	69.9	43.8
3.3	38.8	74.2	73.1	50.4
2.5	41.5	78.7	72.8	55.3
2.0	43.3	75.0	72.4	59.9
—	87.4	—	—	—

TABLE II

FACTOR X DETERMINATION IN HUMAN, BOVINE AND CROSSED SYSTEMS

Coagulation times obtained with human or bovine plasmas and human or bovine thromboplastins (thr. pl.) in a reagent specific for factor X containing 33% human factor VII, 55% bovine factor V, 24% human factor II and 1.4 mg/ml factor I. The figures are the means of 32 estimations. S.D. ≤ 1%.

Plasma concentration (% v/v)	*Coagulation times (sec)*			
	Human plasma		*Bovine plasma*	
	Human thr. pl.	*Bovine thr. pl.*	*Human thr. pl.*	*Bovine thr. pl.*
10	28.2	69.2	35.0	36.8
5	34.0	75.6	42.3	47.9
3.3	36.1	77.0	46.4	52.0
2.5	39.3	79.5	49.6	55.5
2.0	41.6	79.9	51.7	57.6
—	72.8	97.8	—	—

with either bovine or human thromboplastin. Coagulation times slightly shorter than the buffer value are obtained when plasma and thromboplastin come from heterologous sources. Table II shows that the same is not true for a factor X-deficient system. Apparently only the heterologous system consisting of human thromboplastin and bovine plasma does not work here; whereas short coagulation times are observed with bovine thromboplastin and human plasma.

Thromboplastin in this type of test serves at least a double purpose. With factor VII it forms the factor X activator and it provides the lipid–water interface necessary for the formation of prothrombinase from the factors X_a and V_a (ref. 9). The latter function obviously is not species specific as it can be carried out even by synthetic or plant phospholipids; it thus seems that heterologous thromboplastin can function in this stage. The fact that bovine thromboplastin gives long clotting times with human material would then mean that it cannot readily interact with human factor VII. Whether the activation step or the combination step fails cannot be decided from these experiments. The possibility exists that human factor VII, that has already been activated by means other than reaction with homologous thromboplastin, will still combine with bovine tissue thromboplastin so as to form a factor X activator. To test this hypothesis we carried out the following experiments.

Normal human plasma — containing normal unactivated factor VII — is incubated with low concentrations of either human or bovine thromboplastin. The mixture is then subsampled into a factor VII test as described in Table I with the use of either human or bovine thromboplastin. When no calcium ions are added to the incubation mixture, no activation is observed. With 10 m*M* Ca^{2+} added, the incubation mixture coagulates in approximately 3 min. We therefore sought the Ca^{2+} concentration that made the mixture coagulate in about 2 h. This appeared to be 7.5 m*M*. The results are shown in Figs. 1 and 2. Incubation with bovine thromboplastin under no circumstances gave rise to enhanced factor VII activity. Incubation with human

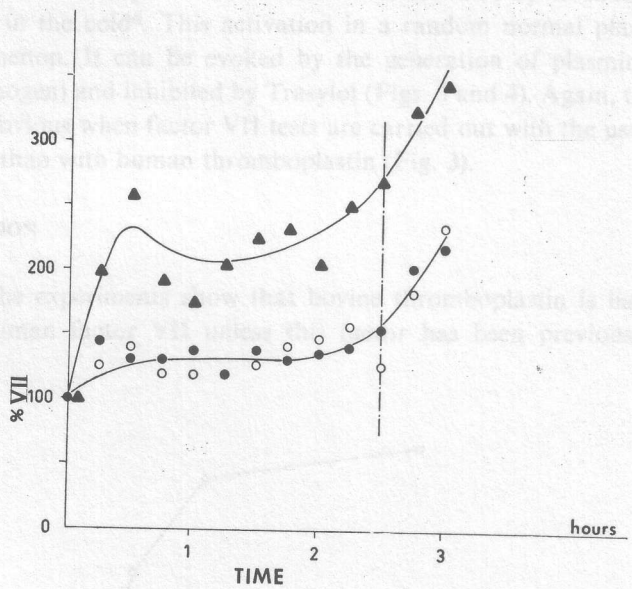

Fig. 1. Estimation of activated factor VII in the course of coagulation. Test mixture: 0.1 ml bovine thromboplastin, 0.1 ml factor VII-deficient plasma, 0.1 ml sample, 0.1 ml $CaCl_2$ (33 m*M*). Incubation mixture: normal human citrated plasma. $CaCl_2$ to 7.5 m*M*, 1% (v/v) of a thromboplastin preparation (*c.q.* blank). ▲, human thromboplastin; ●, bovine thromboplastin; ○, blank (Michaelis buffer). The vertical dotted line indicates the moment of coagulation in the incubation mixture.

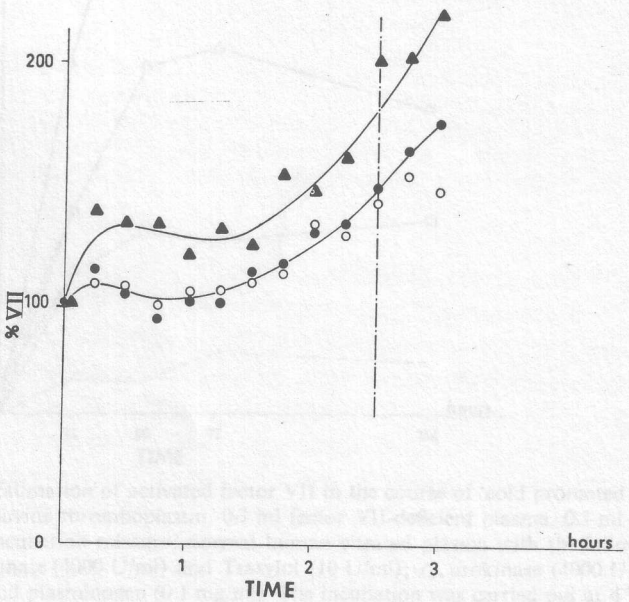

Fig. 2. Estimation of 'total' factor VII in the course of coagulation. Test mixture: 0.1 ml human thromboplastin, 0.1 ml factor VII-deficient plasma, 0.1 ml sample, 0.1 ml $CaCl_2$ (33 m*M*). Incubation mixture: normal human citrated plasma. $CaCl_2$ to 7.5 m*M*, 1% (v/v) of a thromboplastin preparation (*c.q.* blank). ▲, human thromboplastin; ●, bovine thromboplastin; ○, blank (Michaelis buffer). The vertical dotted line indicates the moment of coagulation in the incubation mixture.

thromboplastin caused a slow activation in both test systems. The increase in activity was much more obvious in the test system in which bovine thromboplastin was used than in the human system.

It has been reported that factor VII is activated by incubation without thromboplastin in the cold[6]. This activation in a random normal plasma is an inconstant phenomenon. It can be evoked by the generation of plasmin (*i.e.* urokinase and plasminogen) and inhibited by Trasylol (Figs. 3 and 4). Again, the activation is much more obvious when factor VII tests are carried out with the use of bovine thromboplastin than with human thromboplastin (Fig. 3).

DISCUSSION

The experiments show that bovine thromboplastin is hardly able to interact with human factor VII unless this factor has been previously activated. Bovine

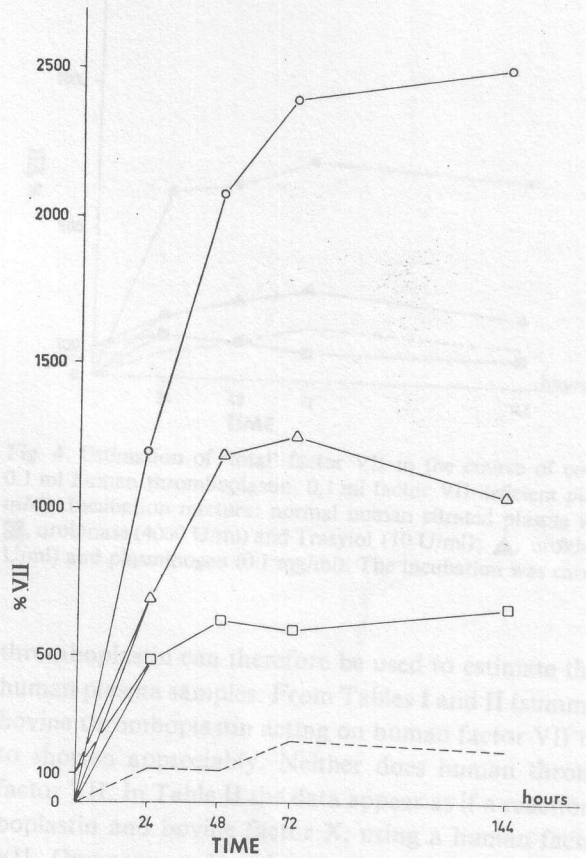

Fig. 3. Estimation of activated factor VII in the course of 'cold promoted activation'. Test mixture: 0.1 ml bovine thromboplastin, 0.1 ml factor VII-deficient plasma, 0.1 ml sample, 0.1 ml $CaCl_2$ (33 m*M*). Incubation mixture: normal human citrated plasma with the following additions. ---, none; □, urokinase (4000 U/ml) and Trasylol (10 U/ml); △, urokinase (4000 U/ml); ○, urokinase (4000 U/ml) and plasminogen (0.1 mg/ml). The incubation was carried out at 4 °C.

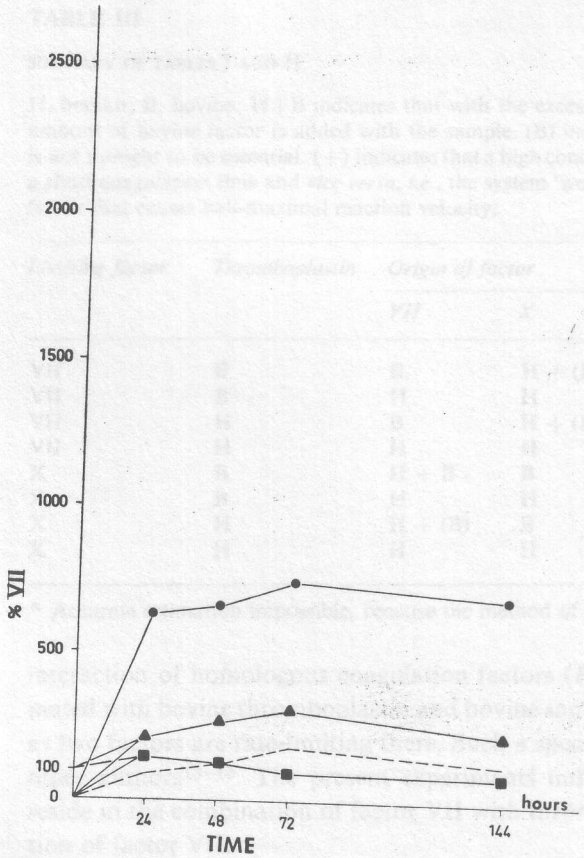

Fig. 4. Estimation of 'total' factor VII in the course of cold promoted activation. Test mixture: 0.1 ml human thromboplastin, 0.1 ml factor VII-deficient plasma, 0.1 ml sample, 0.1 ml $CaCl_2$ (33 m*M*). Incubation mixture: normal human citrated plasma with the following additions. - - -, none; ■, urokinase (4000 U/ml) and Trasylol (10 U/ml); ▲, urokinase (4000 U/ml); ●, urokinase (4000 U/ml) and plasminogen (0.1 mg/ml). The incubation was carried out at 4 °C.

thromboplastin can therefore be used to estimate the level of activated factor VII in human plasma samples. From Tables I and II (summarized in Table III) it is seen that bovine thromboplastin acting on human factor VII does not cause coagulation times to shorten appreciably. Neither does human thromboplastin interact with bovine factor VII. In Table II the data appear as if a reaction is observed with bovine thromboplastin and bovine factor X, using a human factor X reagent, *i.e.* human factor VII. One soon realises however that, with the dilutions of normal bovine plasma meant to be a source of factor X, one adds at the same time bovine factor VII. One thus carries out a combined bovine VII–X estimation. Estimations of the apparent K_m according to Hemker[11] (*i.e.* the concentration of the rate-limiting coagulation factor at which half-maximal reaction velocity is observed under the conditions of the test) showed that although bovine factor VII_a does activate human factor X, the interaction between these proteins is much less efficient ($K_m = 18.0\%$) than the

TABLE III

SUMMARY OF TABLES I AND II

H, human; B, bovine; H+B indicates that with the excess of human factor in the reagent, a small amount of bovine factor is added with the sample. (B) indicates that the presence of bovine factor is not thought to be essential. (+) indicates that a high concentration of the rate-limiting factor causes a short coagulation time and *vice versa*, *i.e.*, the system 'works'. K_m is the percentage of rate-limiting factor that causes half-maximal reaction velocity.

Limiting factor	*Thromboplastin*	*Origin of factor*		*Results*	K_m
		VII	*X*		
VII	B	B	H + (B)	+	18.0
VII	B	H	H	—	—
VII	H	B	H + (B)	—	—
VII	H	H	H	+	1.9
X	B	H + B	B	+	2–5*
X	B	H	H	—	—
X	H	H + (B)	B	+	4.0
X	H	H	H	+	2.1

* Accurate estimation impossible, because the method of estimation fails.

interaction of homologous coagulation factors ($K_m = 1.9\%$). No K_m could be estimated with bovine thromboplastin and bovine sample using a human factor X reagent as two factors are rate-limiting there. Such a species specificity has been observed by other authors[13–15]. The present experiments indicate that this specificity does not reside in the combination of factor VII with thromboplastin but rather in the activation of factor VII.

When human plasma is incubated with a small amount of homologous thromboplastin a slow rise in activity is observed. The time course suggests an enzymatic activation rather than a stoichiometric complexation as the complexation step has been shown to be very rapid[3,4]. The increase in activity is very marked when bovine thromboplastin is used in the test system, suggesting that the factor VII activated by the human thromboplastin during preincubation combines with the bovine thromboplastin. With the use of human thromboplastin in the test system, an increase in factor VII activity can still be observed. This can be readily explained by assuming that the activation step and not the combination step is rate-limiting as would be expected from the observed kinetics of the combination[3,4]. Although suggestive of the idea that activation of factor VII is species specific and combination with the thromboplastin lipoprotein is not, these experiments lack conviction because an effect of combination of factor VII with homologous thromboplastin during the preincubation cannot be ruled out. This is not the case in the experiments shown in Fig. 3. Here factor VII is activated at 4 °C as described by Gjønnaess[6].

The apparent concentration of factor VII, when tested with bovine thromboplastin, is dramatically increased by this type of activation; whereas the increase as observed with the use of human thromboplastin is much smaller. This again argues in favour of the idea that activated factor VII is measured with bovine thromboplastin.

When tested with human thromboplastin, previously unactivated factor VII is activated during the test procedure. This activation is by no means complete, so that the outcome of the normal one-stage procedure using human thromboplastin is influenced by the state of activation of factor VII in the plasma. This may be a source of error in factor VII estimations and it may well be the cause of the reportedly high factor VII concentration in elderly people. The state of activation of factor VII in a sample can be assessed by determining the ratio of the factor VII level estimated with bovine thromboplastin over that estimated with human thromboplastin.

ACKNOWLEDGEMENT

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